JOTTINGS FROM THE BIOLOGICAL LABORATORY OF SYDNEY UNIVERSITY.

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1. On a destructive Parasite of the Rock Oyster.

I was requested some time ago by Dr. Cox, the President of the Fisheries Commission, to examine some samples of oysters from the Hunter River beds, which appeared to be dying in large numbers owing to the attacks of some parasite. On examining the specimens which I received, I found that most of them, when opened, presented on the inner surface of the shell one or more discoloured blisters. In some these raised discoloured patches were of small extent, with a narrow sinuous form, while in many instances a large part of the valve was affected. In some cases, where the extent of the shell invaded was not large, the oysters did not seem at all affected by it; in other cases the animal was found to be dead, and in a few cases the shell was completely empty.

A very slight pressure suffices to break open the blisters, which are covered only by a thin layer of nacreous substance, and their interior is found to be occupied by fine black mud. In the earlier stages, instead of a fair-sized open cavity, there is merely a narrow tunnel bent upon itself, excavated in the substance of the shell, and opening on the exterior at the edge of the valves; but where the mischief has spread further the greater part of the substance of the shell beneath the blisters has become more or less disintegrated and readily splits up into soft laminæ, with often an infiltration of fine mud between them. In almost every instance I found in the interior of the cavity one or more specimens of the little animal by which the mischief had been effected,—a very small annelid of the genus *Leucodore* or *Polydora*.

Species of this genus have been long known as accustomed to burrow in the shells of Molluscs—among others of the oyster—as well as in any sandstone, or shaley, or calcareous rock; but they do not seem to have been regarded generally as serious enemies of the oyster.

Oersted (1) does not mention the boring propensities of *Leucodore* at all, but merely describes it as found on a sandy bottom. Grube (2) describes it as perforating cretaceous rocks at Dieppe. In his "Histoire Naturelle des Annélés," Quatrefages does not allude to the shell-invading habits of the genus, but describes it as either living in delicate tubes, or in burrows in sand or calcareous rock. But MacIntosh, in a paper "On the boring of certain Annelids" published in the Annals and Magazine of Natural History, for 1868, mentions that he had observed *Leucodore ciliata* burrowing in the shells of various molluscs, among others in those of the oyster. I have not been able, however, to find any record of such extensive destruction of oysters effected by this little annelid on the European coast as seems to be taking place on the Hunter River (3), where no doubt some local circumstances, such as muddiness of the water produced by increasing traffic, tend to decrease the vital powers of the oysters and thus favour the inroads of the parasites.

The species which is found most adundantly in these oysters from the Hunter River beds is, strange to say, identical with the European *Polydora ciliata* of Johnston (4).

I found, however one specimen of a second species which appears to be very distinct and of which I append a description.

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(1) Annulatorum Danicorum Conspectus, Fasc. I.

(2) Beschreibungen neuer oder wenig bekannter Anneliden.

(3) Prof. Huxley, in a popular article on the oyster in the "English Illustrated Magazine" of last year, mentions that he had received from Sir Henry Thomson specimens of oysters which were invaded by a species of *Leucodore*, but adds that the oysters seemed little the worse.

(4) Claparèdés *P. Agassizii* is apparently the same as *P. ciliata* (Annélides Chetopodes du Golfe de Naples, p. 314, pl. XXII., fig. 1.)

POLYDORA (LEUCODORE) POLYBRANCHIA. N. S.

The head is of the same breadth behind as the segments of the body. In front it becomes rather narrower, and ends anteriorly in two low triangular lobes separated by a wide notch. There are four very small rounded eyes. Running backwards from the mouth is a narrow groove continued as far as the third segment. The branchiæ begin on the second segment of the body. The large setæ on the fifth segment are ten in number and arranged in two sets which differ from one another in shape and in direction. The five which are directed towards the ventral aspect of the animal end in a broad head having the form of an inverted cone with an oblique base ; on the base of the cone are one or two small conical elevations. The five which are directed more towards the dorsal side are not so broad at the end and are gently curved in the form of a hook with a blunt apex. The ordinary setæ and uncini are precisely similar to those of *P. ciliata* as figured by MacIntosh. The uncini or hooked setæ begin on the seventh segment ; there are from six to ten of them on each parapodium, all with the apex directed outwards, except the most external, which is very short, and has the apex directed inwards. Some of the setæ of the anterior parapodia are very long and filiform.

The setæ are not unlike those figured by Ray Lankester (Ann. Mag. Nat. Hist., (4th series) Vol. I., 1868) as those of *P. calcarea*, and regarded by MacIntosh (1) as belonging to a variety of *Polydora ciliata* : but apart from the form of the boring setæ, the presence of branchiae on all the segments, together with the absence of cephalic tentacles, seems to distinguish the present species from all hitherto described forms. *Polydora cœca* of Oersted (2) resembles it in having "branchias in utroque corporis parte," but has long præstomial tentacles.

The only southern species of *Polydora* described is *P. socialis* of Schmarda (3) which has a pair of well-developed tentacles.

(1) Ann. Mag. Nat. Hist., 1868.
(2) "Zur Klassification der Anneliden." *Vide* Quatrefages, Hist. Nat. Ann. II., p. 302.
(3) Neue Wirbellose Thiere. I., ii., p. 64, pl. XXVII., fig 209.

2. On some recent Histological Methods, and their adaptation to the teaching of practical Histology.

Some methods, recently described, of dealing with objects intended for histological examination are not only great boons to the original worker, lessening greatly the drudgery of manipulations, and enabling him to prepare and examine a large amount of material in a comparatively short time; but are, in certain instances, of great service also to the teacher of histology, as, when perfected, they will enable him to supply the largest class without much loss of time with a uniform series of preparations so preserved and stained as to bring out all the main points in their microscopic structure. A short account of my experience of some of these methods in connection with class work, will perhaps be of service to others who have to do with the teaching of natural science.

STAINING WITH HÆMATOXYLIN.

Objects which have been hardened by any of the usual methods, after having been at least a fortnight in alcohol, are best stained *en bloc* by an aqueous solution of crystallised hæmatoxylin, followed by bichromate of potash as recommended by Heidenhain. (1) For most organs and tissues, pieces half an inch square, are most successfully and uniformly stained through by means of a $\frac{1}{2}$ per cent. solution of hæmatoxylin allowed to act for 10 to 24 hours; the staining agent is followed by a 1 per cent. solution of bichromate of potash, which should be allowed to act for two or three hours. It is quite impossible, I need hardly add, to lay down any precise rule as to the time required for staining satisfactorily portions of any given organ; though twenty-four hours immersion in a half-per-cent. solution of hæmatoxylin will, in the majority of cases, give satisfactorily results, in some instances the object will be rendered too black, and in others will be found not to be stained throughout. The tissues which require the most prolonged staining, when hardened by one method, may

(1) Pflüger's Archiv., XXIV. (1884), p. 468.

become much more rapidly coloured when treated in another way. It will, therefore, be found necessary, in order to insure good specimens of all the organs, to take several pieces of each prepared in different ways and subject them all to the same process of staining, or else, taking several pieces of each specimen, to subject each of them to the action of the staining fluid for a different interval. The results obtained by this method excel, in my opinion, in the definiteness of the cell-outlines, and the distinctness of the differentiation of the tissues any that can be obtained by any of the ordinary process of staining capable of being carried out in a class.

EMBEDDING IN PARRAFIN.

Specimens of animals or of organs stained as above described *en bloc* and afterwards treated with bichromate of potash, require, after soaking for a few minutes in distilled water, to be treated with strong alcohol for several days—absolute alcohol being used for at least the last two days—in order completely to remove the water with which they have become saturated. As in staining so also in the embedding both time and material are saved by preparing a large number of specimens—say twenty or more—at one time. The alcohol is then replaced by chloroform. If the objects are delicate and complicated, this will be very conveniently and thoroughly effected by using some such contrivance as the chloroform box which I employ. This is an oblong brass box divided internally by a vertical partition, which does not reach the bottom, but leaves an opening of three-quarters of an inch, into two compartments. Chloroform with a slight admixture of sulphuric ether is poured into the box until it rises a little above the lower border of the vertical partition. Absolute alcohol is gently poured in by means of a pipette on the surface of the chloroform in one of the compartments; the objects are placed in this, and, as they become saturated with the chloroform, they sink down until they drift through below the partition into the other compartment, which contains only the mixture of chloroform and ether. From this they can be taken out without disturbing the

equilibrium of the alcohol and chloroform Ordinary objects may simply be transferred from absolute alcohol to chloroform and kept in the latter for twenty-four hours, or until saturated. Saturation with paraffin is then effected by the well-known method of Giesbrecht. I use a special water-bath with troughs divided into a number of compartments. To ensure a good result equal parts by volume of chloroform and paraffin (of low melting point) should be used, and the objects should be left in the bath at the temperature of the melting-point of the soft paraffin for about twenty-four hours.

To ensure sufficiently fine and delicate sections to obtain the full advantage of the process of staining described above, Caldwell's or some other good form of automatic microtome must be employed, a coating of soft paraffin round the hard in which the object is embedded being added, according to Caldwell's invaluable process, to prevent curling and secure series if required. Sections so prepared and cut can be kept unaltered for an indefinite length of time.

To prevent the sections breaking up or becoming disarranged during the process of mounting in balsam, it will be found desirable in most cases to fix them down to the slide. For this purpose the best agent is Caldwell's shellac dissolved in creosote, which gives much better results than the gum arabic which I previously used and recommended.

3. Minute Structure of *Polynoë*.

In a short paper in the *Zoologischer Anzeiger*, Jordan has recently described the histological structure of the scales of *Polynoë* as revealed by making series of sections. His figures and description agree exactly with what I find to exist in species which I have examined, except that he omits to notice certain cells which I find in the tissue of the scale. The nerve which enters the elytron ramifies through the scale and the ultimate twigs mostly end in minute processes on the surface. Just before the nerve twig enters this end-organ it passes through a little ganglion. These ganglia are mostly composed (and Jordan represents them

Macropus Tibol. *Mackay*